



■ INSTRUCTIONAL REVIEW: TRAUMA

Blast injuries and heterotopic ossification

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Heterotopic ossification (HO) is perhaps the single most significant obstacle to independence, functional mobility, and return to duty for combat-injured veterans of Operation Enduring Freedom and Operation Iraqi Freedom. Recent research into the cause(s) of HO has been driven by a markedly higher prevalence seen in these wounded warriors than encountered in previous wars or following civilian trauma. To that end, research in both civilian and military laboratories continues to shed light onto the complex mechanisms behind HO formation, including systemic and wound specific factors, cell lineage, and neurogenic inflammation. Of particular interest, non-invasive *in vivo* testing using Raman spectroscopy may become a feasible modality for early detection, and a wound-specific model designed to detect the early gene transcript signatures associated with HO is being tested. Through a combined effort, the goals of early detection, risk stratification, and development of novel systemic and local prophylaxis may soon be attainable.

Keywords: Heterotopic ossification, Blast injury, Combat, Ectopic bone, War wounds, Amputation

Introduction

The term heterotopic ossification (HO) refers to ectopic bone formation in non-osseous tissue. It has been well described in the literature, with the usual causes in civilian orthopaedic practice due to polytrauma in combination with traumatic brain (TBI) or spinal cord injury (SCI),¹⁻⁴ and less frequently with total hip replacement,⁵ oncology,⁶ and internal fixation of acetabular and elbow fractures.⁷⁻⁹ Heterotopic ossification was first described in the medical literature over 1000 years ago,¹⁰ but, until recently, understanding of the genetic and biochemical processes behind HO development has been limited.

HO can cause significant loss of function when it forms adjacent to joints, major blood vessels or nerves, and can complicate the use of prostheses following amputation. In fact, in our war-wounded population, it may be the most significant obstacle to functional mobility and return to an active lifestyle or even active duty, affecting amputee and non-amputee patients alike. Treatment of afflicted patients is difficult and requires a dedicated multidisciplinary team approach. Fortunately, most combat casualties that eventually form HO can be managed conservatively. For those patients who develop persistently symptomatic HO, however, the only curative treatment is surgical excision.

Several factors are associated with increased HO formation, but arguably the most important in both military and civilian trauma is an injury to the central nervous system (CNS). Regardless of the mechanism of injury, TBI and SCI are associated with high rates of HO formation in trauma patient populations.¹¹ However, HO remains relatively rare in the civilian trauma population, even when considering patients with concomitant TBI or SCI. Previous studies investigating heterotopic ossification in civilian trauma have repeatedly demonstrated rates of HO formation after TBI that were substantively below the rates found in combat casualties who have sustained penetrating injuries.^{8,11-14} Steinberg and Hubbard¹¹ described an incidence of HO of 54% in the thighs of patients with TBI and associated femoral fractures that had undergone intramedullary fixation. This is the most frequent site and highest rate of HO formation with concomitant head injury. Garland¹² found that ectopic bone complicated the extremities of between 11% and 20% of patients with TBI and SCI. The formation of HO in the context of an associated head injury has been reported in 20% of forearm fractures,⁸ 52% of femoral shaft fractures,¹³ and 0% of tibial shaft fractures.¹⁴ In contrast, we have reported previously that up to 64% of military blast injuries

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14. ABSTRACT Heterotopic ossification (HO) is perhaps the single most significant obstacle to independence, functional mobility, and return to duty for combat-injured veterans of Operation Enduring Freedom and Operation Iraqi Freedom. Recent research into the cause(s) of HO has been driven by a markedly higher prevalence seen in these wounded warriors than encountered in previous wars or following civilian trauma. To that end research in both civilian and military laboratories continues to shed light onto the complex mechanisms behind HO formation, including systemic and wound specific factors, cell lineage, and neurogenic inflammation. Of particular interest, non-invasive in vivo testing using Raman spectroscopy may become a feasible modality for early detection, and a wound-specific model designed to detect the early gene transcript signatures associated with HO is being tested. Through a combined effort, the goals of early detection, risk stratification, and development of novel systemic and local prophylaxis may soon be attainable					
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Fig. 1a



Fig. 1b

Radiograph (a) and three-dimensional CT rendering (b) of a patient with severe pelvic heterotopic ossification after bilateral hip disarticulations due to combat-related blast injuries.

to an extremity developed HO, with a majority of patients not sustaining associated TBI.¹⁵⁻¹⁷

There is a significant ongoing effort to discover the environmental, biochemical, and genetic causes of HO, and to develop novel local and systemic prophylaxis to combat this debilitating condition. This manuscript will review the causes of and current treatments for HO, as well as presenting the most recent advances on the topic in basic, translational and clinical science.

The military HO epidemic

The military and medical technology deployed to the battlefield during the wars in Iraq and Afghanistan has increased the likelihood of survival following severe injury when compared with previous conflicts. We can reasonably argue that vastly improved personal protective gear and ubiquitous pre-hospital tourniquet use, coupled with advanced, far-forward surgical and resuscitative techniques have enabled severely injured service members to survive devastating extremity injuries that would previously have been fatal.¹⁸⁻²⁰ In fact, when applied appropriately, these battlefield medical advances allow approximately 90% of combat casualties to survive their injuries.¹⁹ The epidemic of blast-associated extremity injuries sustained in the current conflicts and subsequent high rate of HO formation are thought to be related (Fig. 1).

Although HO has been reported in the United States Military medical literature since the Civil War,^{21,22} we have previously reported an exceptionally high rate of post-traumatic HO formation in veterans of the recent conflicts in Iraq and Afghanistan.¹⁵⁻¹⁷ In those studies, it was found that between 63% and 64% of combat casualties that met the inclusion criteria developed radiologically evident HO. Several variables were found to be associated with both HO formation and severity on univariate analysis, including

blast mechanism of injury, amputations within the initial zone of injury, and TBI. On multivariate analysis, an Injury Severity Score (ISS)²³ of ≥ 16 , age < thirty years, an amputation (especially if performed within the zone of injury) and multiple extremity injuries were independently associated with HO formation.¹⁵⁻¹⁷ Of particular importance is the presence of an amputation, which until recently, was not thought to be associated with HO formation.

Another factor likely to contribute to the formation of HO in combat casualties is the relationship between residual wounds and the zone of injury. We place significant emphasis on preservation of residual limb length and functional joint levels whenever reasonably practicable. This, coupled with an aggressive limb salvage strategy for other severely injured, non-amputated limbs, leads to most wounds being closed within the zone of injury.

Prophylaxis and treatment

The only medication for treatment or prevention of HO in the United States approved by the Food and Drug Administration (FDA) is the first-generation bisphosphonate etidronate, but the efficacy of this treatment is questionable and it may simply delay HO mineralisation.²⁴ For civilian patients at risk for developing HO, there are therefore two main practicable prophylactic treatments available that have been demonstrated to be essentially equally effective: low-dose radiation therapy (most commonly 7 Gy given in a single fraction) and long-term oral non-steroidal anti-inflammatory drug (NSAID) therapy.²⁵⁻³⁵ Multiple studies and recent meta-analyses have demonstrated the efficacy of both radiotherapy and NSAID treatment; however, patient compliance and gastrointestinal irritation leading to discontinuation may limit the effectiveness of NSAID therapy in some patients.^{15,16,25-36} Unfortunately, there are several side



Fig. 2a



Fig. 2b

Figure 2a – pre-operative photograph of a medial thigh ulceration in a limb salvage patient with heterotopic ossification (HO) enveloping his femoral vessels and causing secondary knee arthrofibrosis. Figure 2b – clinical photograph of HO ulcerating through the distal aspect of a long transfemoral amputation. Note the skin graft over the terminal portion of the residual limb. Although we advocate avoiding terminal skin grafting of residual limbs whenever practicable, due to the limited available soft-tissue envelope, we are sometimes forced to cover portions of residual limbs with split thickness grafts. Unfortunately, this patient failed conservative therapy and required eventual surgical excision with concomitant resection of the overlying skin graft.

effects, limitations, and complicating factors that make these current prophylactic measures unsuitable for carte blanche utilisation following combat-related injuries.

Radiotherapy is not appropriate for combat-related injuries. There are several contraindications to its use in these patients, including severe systemic polytrauma, open and contaminated wounds requiring serial debridements, and fractures or spine injuries requiring operative stabilisation and fusion. The infrastructure needed to provide radiation therapy is also not logistically feasible in the forward combat-support environment, relegating it to larger, tertiary care medical facilities. Importantly, since current recommendations for HO prophylaxis require delivery of radiotherapy within 48 hours of injury,^{26,29,30} most combat casualties would not receive treatment within the appropriate window.

NSAID therapy, while logistically feasible, carries its own set of contraindications in combat-wounded patients. Increased risk of bleeding, delayed fracture healing, impaired renal function and gastritis are all significant relative contraindications. While this is generally true for traditional NSAIDs, celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor, appears to have a relatively well-tolerated side effect profile, and has been shown to prevent HO formation following surgeries of the hip and acetabulum.³⁷⁻³⁹ In fact, we are currently enrolling combat casualties in a prospective, randomised controlled trial to evaluate the effectiveness of COX-2 inhibitors on prevention of HO formation, as well as to assess the adverse side effects in this patient population, including bleeding and delayed long bone fracture healing.⁴⁰

While the risks associated with NSAID and radiotherapy prophylaxis will not change, the best possible practice for

their use in combat casualties is to develop a way to selectively treat patients that are at greatest risk of developing severe HO. Routine treatment for every service member with an extremity injury or amputation is obviously not appropriate based on the aforementioned side-effect profiles of the available therapies. Although advances have been made as discussed below, the authors are aware of no accurate, reproducible means that can reliably portend the eventual development of HO in such patients. Better means of risk stratification are needed to both limit the complications associated with primary prophylaxis, but also as a framework to test new local and systemic therapies currently in development. To this end, the biological process by which HO forms is the subject of research at both military and civilian institutions.

Regardless of the cause of HO, once function has become limited, the only therapy shown to be effective is surgical excision. Our algorithm for treating symptomatic HO begins with an exhaustive trial of conservative therapy. This includes rest, adjustment of pain medication, selected injections and nerve ablations for HO associated with neuromata, and multiple attempts at socket modification for amputees, as well as physical therapy for patients in whom HO is functionally limiting with regard to pain or range of movement. Only patients that fail conservative therapy are candidates for excision (Fig. 2). Fortunately, the rate of persistently symptomatic HO requiring excision is relatively low (approximately 25% of affected amputees), even in our patient population.¹⁶ We have previously reported on the prevalence and risk factors for developing HO in combat casualties,¹⁶ as well as our early experience with and procedures for HO excision from residual limbs. We generally wait an approximate

minimum of six months prior to performing an excision. This gives complex wounds a chance to heal and the ectopic bone time to adequately corticate and mature, which greatly eases dissection and marginal excision while ostensibly minimising the recurrence risks. Currently, we routinely use celecoxib³⁷ for post-excision HO secondary prophylaxis, but only use peri-operative radiotherapy in the most severe cases due to formerly mentioned concerns for wound healing and our previously reported wound complication rate after excision approaching 25%.¹⁶ Of note, we do not routinely use Technetium-99 scintigraphy or serum alkaline phosphatase to determine maturity of the lesion(s). To our knowledge, we have not had any recurrence of symptomatic HO in well over 100 excisions using this algorithm, including some patients with TBI.

State of the art: current research into combat-related HO

It has been hypothesised that HO is caused by both systemic and wound-specific responses to trauma. Current research into HO can be divided into two main areas: 1) identification of the progenitor cells suspected of causing HO; and 2) investigating the biochemical environment that drives osteogenic differentiation of these progenitor cells both systemically and locally. Based on our current understanding of HO formation, we believe that dysregulation of the systemic and local inflammatory system, blast mechanism of injury, delayed wound healing and bacterial colonisation all play a significant role in combat-related HO formation. As mentioned previously, early risk stratification based on wound specific or systemic inflammatory markers is one key area of investigation. In their study of wound effluent and serum cytokines, Evans et al⁴¹ confirmed that an elevated ISS ($p = 0.006$) is associated with HO formation. They also demonstrated that bacterial colonisation and impaired wound healing were both associated with ectopic bone growth ($p < 0.001$ and $p < 0.005$, respectively). Interestingly, serum (IL6, IL10, and MCP1) and wound effluent (IP10 and MIP1 α) were individually associated with HO development (both $p < 0.05$), shedding new light on the role that post-traumatic inflammation plays in the eventual development of HO.

As Evans et al⁴¹ demonstrated in their cytokine study, impaired wound healing is a significant factor in HO development. With that in mind, one of the most promising areas of research involves advanced imaging. Raman Spectroscopy allows for non-destructive and non-invasive *ex vivo* and *in vivo* study of wound conditions at various stages of the healing process.⁴² By looking at the vibrational bands specifically associated with the chemical bonds within biological molecules linked with wound healing, Crane et al⁴³⁻⁴⁵ demonstrated signs of ossification and mineralisation very early in the formation of heterotopic bone. Critically, they were also able to show evidence of decreased collagen deposition in wound

beds by comparing wounds that healed with those that eventually went on to dehisce.⁴³ By obtaining this data early in the wound debridement process, we will be able to determine in real time not only those wounds that are likely to heal, but also those wounds that are more likely to form heterotopic bone. This may be useful in developing a wound-specific method of risk stratification, and perhaps combined with other physiological, cellular, histopathological and/or molecular characteristics as part of a multi-modal clinical decision support model.

As described previously, there is a strong correlation between CNS injury and HO formation. Salisbury et al⁴⁶ recently published data that demonstrate a significant correlation between injury to the peripheral nervous system (PNS), subsequent neurogenic inflammation and HO formation. In their experiment, mice without functional sensory nerves had significantly reduced amount of quantified HO formation ($p \leq 0.05$).⁴⁶ The authors attributed this to the decreased expression of substance P (SP) and calcitonin gene-related peptide (CGRP), which contribute to neurogenic inflammation by recruiting mast cells. Likewise, Rodenberg et al⁴⁷ demonstrated local metalloproteinase-9 (MMP-9) elevation *in vivo* 48 hours after induction of HO in their murine model using micro-positron emission tomography. This preliminary data may be useful in a future prognostic model if MMP-9 is differentially expressed in war wounds that eventually develop HO. A more accurate rat model that reflects the types of wounds sustained by combat casualties, and therefore similar types of HO, will allow future testing of MMP-9, and several other gene products.

While significant progress has been made deciphering the biochemical milieu associated with the development of HO, the role of the progenitor cells is also being investigated. Recently, our group reported that military service members who sustained high-energy wartime injuries had significantly more muscle-derived connective-tissue progenitor (CTP) cells per gram of tissue than non-injured controls ($p < 0.0001$).⁴⁸ Although wounds had increased quantities of progenitor cells committed to a connective tissue phenotype, these cells were not yet further committed to a bone.⁴⁸ This effect was also confirmed by Jackson et al,⁴⁹⁻⁵¹ who demonstrated that muscle-derived progenitor cells present in extremity blast wounds are multipotent and possess the capability to differentiate into osteoblasts, chondrocytes and adipocytes. Therefore, it seems possible that timely intervention may derail this osteoblastic potential in favor of other, more favorable, mesenchymal phenotypes such as muscle, nerve or fat.

Shimono et al⁵² were able to prevent HO formation in a murine Matrigel/rhBMP-2 model by targeting chondrogenesis with a highly selective synthetic retinoic acid receptor-gamma agonist (RAR- γ). They went further to demonstrate that mouse mesenchymal stem cells, when treated with RAR- γ agonist *in vitro*, lost the ability to differentiate into osteogenic cells.⁵³

While there are currently several excellent rodent models that reliably produce HO, they are largely dependent upon exogenous induction, often via injections involving BMP-2 or BMP-4, which we do not feel mimics the same conditions under which HO forms in combat injuries. Tannous et al⁵⁴ described a blast-amputation model that was able to consistently produce HO in rat residual limbs. While their technique has potential, possible variations in the blast overpressure delivered to the animal, failure to quantify the systemic inflammatory insult, as well as high mortality using this technique, may limit its applicability. Systemic blast exposure and contamination of the open wounds with both local and hospital-acquired microorganisms is ubiquitous in combat-wounded patients, and likely play a vital role in inflammation and subsequent HO formation in these patients. We are also currently developing a rat model that will, as much as possible, re-create the physiologic insult sustained by combat casualties. These include a reproducible systemic blast overpressure, open fractures, induced wound ischaemia creating a large zone of injury, introduction of an appropriate bioburden, and amputations through the zone of injury.

Conclusions

Combat-related and blast-induced HO is exceedingly common and often clinically problematic. The aetiology of blast-induced HO is extremely complex, and our current understanding, while having made great strides recently, remains somewhat rudimentary. HO formation is likely mediated by the complex interaction of both systemic and local wound inflammation, including activation and differentiation of CTPs, likely recruitment of circulating progenitor cells, over expression or suppression of key genes, and blast-related injury mechanism; all of these collectively leading to an intricate cascade of events that eventually produces mature, lamellar bone in non-osseous tissue. While novel prophylactic therapies are the ultimate goal, wound specific-risk stratification of severe extremity injuries would allow current therapies to be employed more judiciously in hopes of increasing patient function and preventing future surgical excisions while minimising prophylaxis side effects.

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of the Army, Department of Defense, nor the U.S. Government.

We certify that all individuals who qualify as authors have been listed; each has participated in the conception and design of this work, the analysis of data, the writing of the document, and the approval of the submission of this version; that the document represents valid work; that if we used information derived from another source, we obtained all necessary approvals to use it and made appropriate acknowledgements in the document; and that each takes public responsibility for it.

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- K. A. Alfieri: Principal author: formal manuscript draft preparation, relevant literature search and review (data collection)
- B. K. Potter: Senior author: drafted proposed manuscript outline (study design), reviewed and edited manuscript repeatedly including final manuscript draft
- J. A. Forsberg: Head orthopaedic scientist for HO-related projects at our affiliated lab, revision of manuscript for scientific content and merit (data analysis)

ICMJE Conflict of Interest:

- None declared

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